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Brief/Technical Note

Effect of Surfactants and Solutes (Glucose and NaCl) on Solubility of Kavain—A Technical Note

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INTRODUCTION

Micelles are self-assembling aggregates formed by surfactant molecules in the liquid media above the critical micellar concentration (CMC) value (1,2). In water medium, the internal portion of micelles is a "hydrophobic core" which can host the drug and increase its solubility in water, its bioavailability and minimize its degradation and loss (3–9).

Kavain (Fig. 1) is the most representative kavalactone of the structurally related constituents of *Piper methysticum* G. Forst. (kava-kava), a tropical plant growing in the South Pacific Islands (10) and traditionally used for its soporific and narcotic effects (10,11). Kava-kava extracts or isolated kavalactones are nowadays used for the treatment of anxiety and improvement of stress disorders, nervous tension and restlessness (10) and recently, a local anaesthetic activity has also been reported (10–12). Thus, in view of a possible therapeutic use of kava-kava extracts or single constituents for local anaesthesia, the behaviour of kavain, tested as a model-molecule, in the presence of micellar carrier systems, relatively unexpensive and simple formulation to topical application, was investigated in order to improve its scarce water solubility. Studies were carried out using different types and concentrations of surfactants (Sodium dodecyl sulfate, SDS, sodium lauryl ether sulfate, SLES and octanoyl-6-*O*-ascorbic acid, ASC-8, Fig. 1) and physiological solutes (glucose and NaCl).

SDS is an anionic surfactant that is used in household products such as toothpastes, shampoos, shaving foams and bubble baths for its thickening effect and its ability to create a lather; SLES is a detergent and surfactant found in many personal care products, it is an inexpensive, very effective foamer, very stable at normal pH ranges (13). Besides behaving as a surfactant, with a low CMC, ASC8 retains the antioxidant properties of vitamin C and acts as a powerful radical scavenger both in aqueous and in non-aqueous media. It is employed in food industry, it is under consideration for human use and should be suitable both for oral and parenteral formulations (14,15).

Several geometrical parameters and some descriptors (4,16) related to micelles were also investigated in order to describe the structure and properties of the micellar systems.

MATERIALS AND METHODS

Materials

Kavain (99% HPLC) and Sodium dodecyl sulphate (SDS) was from Sigma-Aldrich (Milan, Italy). Octanoyl-6-*O*-ascorbic acid (ASC-8) was synthetised according to literature (17). Sodium laurylethere sulphate (SLES) 28% *w*/*v* in water was from Galeno (Prato, Italy). The 0.9% NaCl and solution of glucose 10% were from Fresenius (Verona, Italy). HPLC grade MeOH and 98% formic acid were provided by Merck (Darmstadt, Germany). Water was purified by a Milli-Q_{plus} system from Millipore (Milford, MA, USA).

Methods

Sample Preparation

Micellar systems were prepared at 25 °C by dissolution each surfactant in pure water or physiological solutions. The micellar solutions were saturated with kavain and sonicated for 15 min. Before HPLC injection, samples were filtered and diluted 1:25 in HPLC grade MeOH. CMC values of surfactants in water were taken as reference from literature, as SDS (18), ASC-8 (19) and SLES (20). All the surfactants were investigated using increasing concentrations, i.e. from CMC to 120 or 150 mM.

HPLC Analysis

Kavain concentration was determined by HPLC/DAD (High-performance liquid chromatography-diode array detector) analysis from the mean of three determinations. A calibration curve was obtained from 0.001 to 1.5 mg/ml of kavain in methanol.

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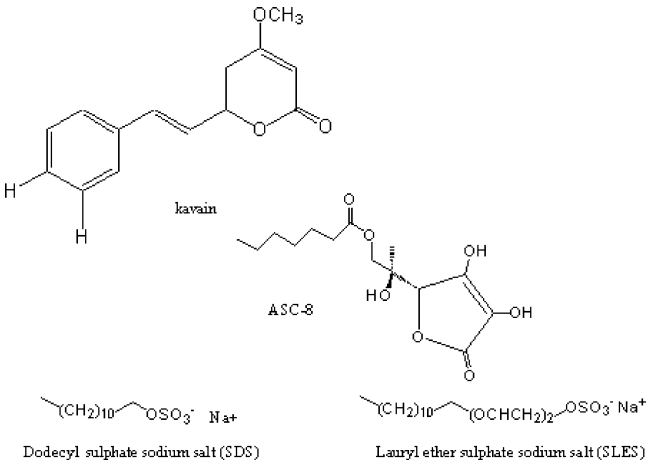

Fig. 1. Structures of kavain and of the tested surfactants

The HPLC system is a HP 1100L instrument with DAD controlled by a HP 9000 workstation (Hewlett and Packard, Palo Alto, CA, USA). The column was a Phenomenex Prodigy ODS3 (5 μm, 150×4.6 mm) maintained at 40 °C. The injected volume was 15 μl and the chromatograms were monitored at 254 nm. Flow was 0.2 ml/min. Solvents were MeOH and H_2O (pH 3.2), 50:50 for a run time of 30 min.

Calculation of Geometrical Parameters and Descriptors of the Micellar Systems

The volume (V_H) and length (L_{HC}) of the hydrophobic chain of each micellar system were calculated according to Tanford (2) by the equations:

$$L_{HC} = 1.5 + 1.265 n_c \ (\text{Å}) \tag{1}$$

$$V_H = [27.4 + 26.9(n_c - 1)] \ (\text{Å}^3) \tag{2}$$

where n_c was the number of carbon atoms of the hydrocarbon chain of each surfactant

Consequently, total core surface (A) may be calculated as:

$$A = 4\pi L_{HC}^2 \ (\text{Å}^2) \tag{3}$$

Thus, the base-area of conical fully-extended surfactant-units A_m can be considered as the core area of the monomer, from the ratio $A/\overline{n}$. Approximating this area to a circle, the related diameter d could be considered as a simple approximation of the distance between hydrocarbon chains at the level of core surface.

$$d = 2 \times \sqrt{\frac{A}{\overline{n}\pi}} \ (\text{Å}) \tag{4}$$

Critical packing parameter $P_C = V_H/a_0 \times L_{HC}$ can also be calculated according to Isralachivili *et al.* (21,22), being P_C the area of the polar head of the surfactant monomer (a_0) to determine the most presumable shapes of surfactant associates.

To quantitatively differentiate the solubility power of the three surfactants the molar solubilization capacity (k) of the surfactant and the number of kavain molecules dissolved in one micelle (n_m) were evaluated according to Alvarez-Núñez and Yalkowsky (4) and Edwards *et al.* (16).

According to Alvarez-Núñez (4), the apparent solubility of kavain S_t is related to the concentration of surfactant molecules in water by the equation:

$$S_t = S_0 + k(C_{surf} - \text{CMC}) \tag{5}$$

where S_0 is the solubility of kavain in water, C_{surf} is the total concentration of surfactant and CMC is the critical micellar concentration of surfactant. The k value, i.e. the number of moles of the solute which can be dissolved by 1 mol of surfactant above CMC, is obtained by the slope of the curve $S_t - S_0$ *vs.* ($C_{surf} - \text{CMC}$).

The second method to evaluate k is derived from Edwards *et al.* (16), as follows:

$$\text{MSR} = k = \frac{S_t - S_{CMC}}{C_{surf} - \text{CMC}} \tag{6}$$

where S_{CMC} is the solubility of kavain at CMC.

Finally, it is also possible to obtain the molar ratio of kavain dissolved in micelles (n_m), respectively from (4):

$$n_m = \frac{S_t - S_0}{M} \tag{7}$$

while from (16):

$$n_m = \frac{S_t - S_{CMC}}{M} \tag{8}$$

where M is the concentration of micelles in solution obtained from:

$$M = \frac{(C_{surf} - \text{CMC})}{\overline{n}} \tag{9}$$

RESULTS AND DISCUSSION

Evaluation of Kavain Solubility in Micellar Systems

The solubility of kavain in water was 16 μg/ml at 25 °C, evaluated by HPLC/DAD analysis. The same HPLC analysis was also applied for evaluating the apparent solubility of kavain in the different micellar systems. In the presence of micelles the concentration of kavain increased remarkably and linearly, as reported in Fig. 2. At the maximum surfactant concentration tested, the observed increment of kavain solubility was about 1,600 times in the presence of SDS micelles, while it was about 1,100 times in the presence of SLES micelles and about 160 times with ASC-8 micelles.

Geometrical Parameters and Descriptors of the Micellar Systems

All the investigated micelles are aggregates of short-chain surfactants and can be considered spherical, with the fully-extended surfactant-units in a conical shape. Several

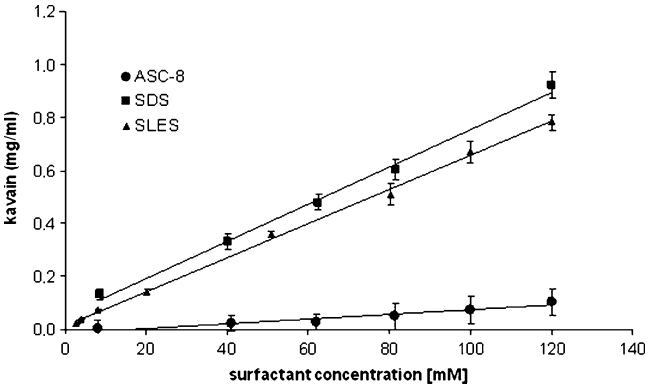

Fig. 2. Solubility of kavain in SDS (*filled square*), SLES (*filled triangle*) and ASC-8 (*filled circle*) micellar solution

geometrical constrains were calculated to justify the different solubilities of kavain (Table I) and other parameters were found in the literature (23,24).

V_H and L_{HC} were calculated according to Tanford (2) and are reported in Table I with the values obtained for P_C and V_H.

Through evaluation of these parameters it was possible to justify the different solubility of kavain within the three micellar systems. Thus, even if L_{HC} of SDS and SLES are the same, the resulting V_H differs dramatically due to the different distances between hydrocarbon chains (d). The greatest values of d obtained within SDS and SLES micelles can be explained considering the electrical properties of polar heads of the two surfactants, due to the negative charge, and as a consequence the hydrophobic chains of these surfactants are more distant compared to those of ASC8. In addition, SLES micelles are the most compact due to the presence of oxyethylenic groups in the monomers.

Table I. Known Geometrical and Calculated Properties of SDS, SLES and ASC-8 Micelles According to the Literature Data (in Brackets)

Parameter	Acronym	SDS	SLES	ASC-8
Average aggregation number of surfactant in micelles	$\bar{n}$	64 (4)	86 (21)	80 (16)
Total micellar radius (Å)	R_{HC}	23.3 (20)	26.4 (21)	25.4 (5)
Area of the polar head of the surfactant monomer (Å^2)	a_0	52.5 (20)	63.2 (17)	76.0 (5)
Critical micellar concentration [mM]	CMC	8.1 (4)	2.9 (17)	8.2 (16)
Length of the hydrophobic chain (Å)	L_{HC}	16.7	16.7	11.7
Distance between hydrocarbon chains (Å)	d	8.3	7.2	5.2
Volume of the hydrophobic chain (Å^3)	V_H	299.2	195.3	73.6
Critical packing parameter	P_C	0.34	0.19	0.08
Hydrophilic portion of micelle	$R_{HC}-L_{HC}$	6.6	9.7	13.8

Table II. Values of k and Calculated Values of n_m, According to Equations Reported in the Literature

	SDS	ASC-8	SLES
k (4)	5.7×10^{-2}	3.1×10^{-3}	3.8×10^{-2}
k (16)	4.5×10^{-2}	2.9×10^{-3}	2.8×10^{-2}
n_m (4)	2.88–3.94	0.18–0.28	2.47–2.90
n_m (16)	2.88–3.92	0.09–0.28	2.45–2.87

k (4) and n_m (4) represents molar solubilisation capacity calculated according to Alvarez-Nunez and Yalkowsky (4); k (16) and n_m (16) the value according to Edwards *et al.* (16)

The characteristics of the polar heads deeply influence the properties of the hydrophilic shell of micellar systems too. As reported in Table I, the hydrophilic portion of micelles ($R_{HC}-L_{HC}$) is more extended in ASC-8 than in SLES and SDS ones: this means that the ASC-8 hydrophilic head interacts deeply with water than SLES and SDS ones. This is also in agreement with the a_0 values which represent the polar head group area of the surfactant monomer in micelles considering the total dimension of the polar head. Thus, the thick hydrophilic shell of ASC-8 micelles could interfere with kavain penetration in the micellar core.

Therefore, the better solubility of kavain in SDS micelles can be explained in terms of more available hydrophobic space to include kavain molecules with respect to SLES and ASC-8 ones and less extension of the hydrophilic shell of SDS polar heads, compared to SLES or ASC-8 polar ones.

These data are reflected in the differences between the regression curves of Fig. 2. The solubility of kavain increases linearly with increasing surfactant concentration. This behaviour results from association of the kavain molecules with the micellar aggregates. The anionic surfactant SDS and exhibited the best kavain solubility profile, probably due to the volume of hydrophobic core which results bigger for SDS and can provide an enough space to accommodate kavain molecules inside surfactant aggregates.

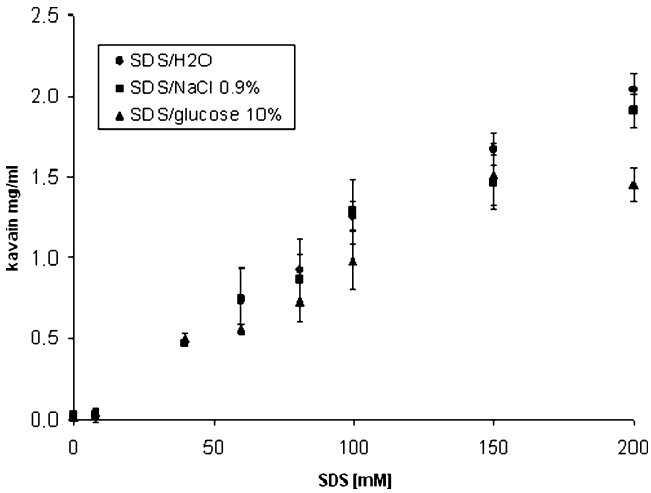

Fig. 3. Solubility of kavain in SDS micellar solution in H_2O (*filled circle*), H_2O/NaCl 0.9% (*filled square*) and H_2O/glucose 10% (*filled triangle*)

HPLC results are also in good agreement with the values obtained with the molar solubilization capacity (k) of the surfactant and the number of kavain molecules dissolved in one micelle (n_m) reported in Table II. SDS solubilization capacity is almost ten times greater than that of ASC-8, while it is of the same order of magnitude of SLES capacity factor, which results lightly lower.

Effects of Physiological Solutes on Kavain Solubility in SDS Micelles

In order to use micellar carriers containing kavain as local anaesthetic (12), the influence of physiological solutes (NaCl 0.9% *m*/*v* and glucose 10% *m*/*v*) on kavain apparent solubility was investigated in the case of SDS micellar systems, those having the best characteristics in solubilizing kavain. Our studies evidenced that the solubility of kavain is not significantly affected till to 200 mM SDS micellar solutions. At this concentration the kavain solubility is slightly decreased in the presence of 10% glucose and no statistically affected by NaCl, when compared to pure SDS solutions (Fig. 3).

Indeed, the properties of micellar systems strictly depend on the properties of the liquid media in which they are dissolved. Salt addition in the solution of ions surfactants modifies their CMC, but generally doesn't affect the shape and the size of micelles (25). Also in the case of sugars, the CMC slightly decreases where as the aggregation number increases (26) and the carbohydrates involve solvatation changes in head groups or counterions (27). The presence of these solutes can produce modifications in water structure, reduction of dielectric constant, modification of the coulombic repulsions between the charged head groups and alters their aggregation and micellar structure.

For ionic micelles, the involved thermodynamic parameters are complex since both hydrophobic and electrostatic interaction are involved in the interaction process. For nonionic surfactants the thermodynamic parameters are obviously less complex.

SUMMARY & CONCLUSIONS

ASC-8, SLES and SDS micelles can enhance the solubility of kavain and they can be considered useful for pharmaceutical application being SDS is the best surfactant. The surfactants had a similar CMC, total micellar radius and length of the hydrophobic chain but they strongly differ in the volume of the hydrophobic chain and the hydrophilic portion of micelle. SDS micelles had the highest volume (299.2 Å, about four times more than ASC-8) with the lowest hydrophilic portion (6.6, about an half of ASC-8) which strongly affected the inclusion of kavain, a very unpolar molecule, resulting in highest apparent solubility with respect to the other micellar systems.

The effects of physiological solutes such as NaCl or glucose in SDS on the kavain solubility was also investigated for a possible pharmaceutical application of such preparation. It has been found that the presence of these solutes does not affect significantly the solubility of kavain in micellar systems, compared to the solubility in SDS systems. The effect of glucose is present at concentrations of SDS 200 mM and could be related to the decrease of the CMC value of SDS, increasing the aggregation number and as a consequence a less efficient encapsulation power of micellar systems toward kavain.

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